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# PROPERTIES OF HUMAN SEMINAL PLASMA.

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THE possibility that seminal plasma may have functions other than those of nutrition of the spermatozoon and of mechanically sweeping out the latter from the urethra is suggested by recent work of v. Euler [1934] and Goldblatt [1933]. The former worker employed human seminal fluid whilst the latter used both the native fluid and extracts made with aqueous alcohol and aqueous acetone. In this communication the pharmacological properties of human seminal plasma are described and a comparison made between the substance or substances responsible for them and other pharmacologically active bodies.

## METHODS.

Seminal fluid was obtained from a considerable number of men, centrifuged and the supernatant, almost cell-free fluid or plasma used for the following experiments. Extracts of seminal plasma were prepared by addition of four volumes of absolute alcohol (or acetone) to one volume of plasma; after thorough shaking the precipitate was removed by filtration. The acetone or alcohol was removed by distillation at 42° C. under reduced pressure. The white residue was taken up in distilled water and made up to the original volume of plasma. This fluid was turbid, alkaline and contained a trace of proteose; it will be referred to as seminal extract. Both seminal fluid and extract were tested for their effects on the blood-pressure of cats and rabbits under ether with and without preliminary treatment with atropine; on the isolated small intestine of the rabbit with and without atropine; on the isolated uterus of the virgin guinea-pig; on the isolated seminal vesicle of the guinea-pig and on the eserinized rectus abdominis of the frog.

### Effect on blood-pressure.

Intravenous injection of seminal fluid or extract is always followed by a rapid fall in blood-pressure, and this fall is usually of great proportions even with very small doses. Subcutaneous injection does not produce any noticeable depression. The intravenous injection of a volume of extract equivalent to 0·25 c.c. seminal fluid produced in one cat (urethane)

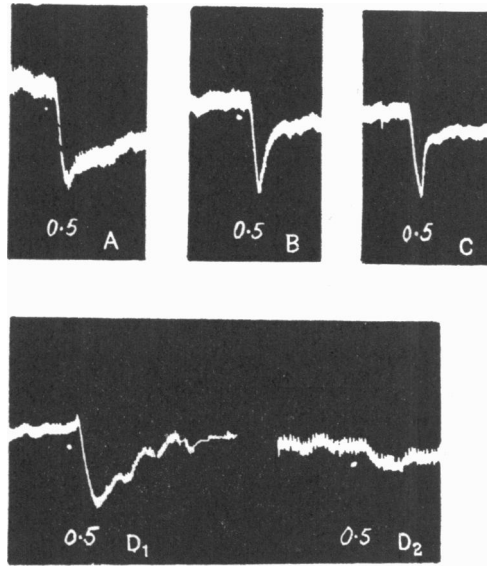


Fig. 1. Cat. Ether, vagi intact. 1 c.c. water-alcohol extract of seminal plasma equivalent to 0·6 c.c. plasma. All injections intravenous. A, 0·5 c.c. extract; B, 0·5 c.c. after heating with three drops fresh $HNO_2$; C, 0·5 c.c. after heating with six drops fresh $HNO_2$; $D_1$, 3 mg. atropine sulphate intravenously and 5 min. later 0·5 c.c. extract; $D_2$, 0·5 c.c. after boiling for 30 sec. with four drops 0·5 *N* NaOH.

a fall of 70 mm. Hg, and in another (ether) a fall of 90 mm. Hg. The depression lasts for a considerable time, and sometimes recovery to the original level of pressure is not attained for 30 or 45 min. The depressor effect is equally marked in the pithed atropinized cat, so that the effect is not a central one. Fig. 1 shows that the depressor is active in the atropinized animal, that it is destroyed by heating for a few seconds in dilute alkali, and that it is not destroyed by heating with nitrous acid. It thus appears that the effect is not due to choline or choline esters or histamine. Further evidence that we are not dealing with histamine was

obtained from the effects on the atropinized rabbit under ether. It is well known that histamine produces a rise in blood-pressure in the rabbit deeply under ether; in conditions, however, in which the pressor action of histamine was readily demonstrable the depressor action of seminal extract was still strong. Treatment with phosphotungstic acid does not precipitate the active substance from extracts but does so from the native plasma; the latter fact is probably due to adsorption on the protein precipitate and can be shown to occur with ammonium sulphate precipitation of the proteins, whilst the same reagent has no precipitating effect on the extracts. We are at some pains to establish that the active substance is not histamine because every extract we have prepared has given a definite and sometimes a marked Pauly reaction. This might be regarded as evidence of the presence of histamine, but if the seminal extract be boiled with an equal volume of dilute alkali, cooled and neutralized, the Pauly reaction is still positive but the depressor action is completely absent. Histamine treated in the same way retained both the Pauly reaction and the depressor effect.

After boiling the seminal extract with an equal volume of 0·1 *N* HCl the depressor activity is found to be diminished but not destroyed even after 5 min. boiling. Like histamine the depressor body in seminal fluid and extracts can be removed by adsorption with charcoal and will readily dialyse through collodion membranes.

The dialysate from seminal plasma is clear, yellowish and alkaline; it gives a positive Pauly reaction, positive Sakaguchi and Millon reactions and a faint pink biuret reaction; it contains proteose. Ultrafiltration gives a similar solution. In one case ultrafiltration at 50 mm. Hg pressure gave a clear yellowish filtrate, 0·5 c.c. of which injected into a cat under ether gave a depression of 80 mm. Hg.

Since both the extracts and ultrafiltrates contain a certain small amount of proteose it was necessary to see if proteoses in small quantities have any depressor effect. Intravenous injection of 2–10 mg. of Witte's peptone into cats under ether produced no depressor effect; rather the reverse occurred, 10 mg. producing in one case a rise of blood-pressure of about 20 mm. Hg which was maintained for several minutes.

The evidence thus far does not eliminate the possibility of choline derivatives being mixed with other depressor bodies. This was done in two ways. First of all neither the seminal plasma nor the extracts produced any effects on the rate or force of the beat of the isolated perfused frog's heart; secondly, the depression of blood-pressure of rabbits under ether was exactly the same before and after treatment with atropine. It

appears, therefore, that seminal fluid is free from choline at any rate after leaving the body.

Although other evidence was obtained that the depressor is not adenosine, it may be mentioned here that adenosine is precipitated by phosphotungstic acid and is not destroyed by boiling in dilute alkali. That the active substance is not similar to the substance described by Frey, Kraut and Schultz [1930] is clear from the facts that the latter is insoluble in 80 p.c. alcohol and is not dialysable. The interesting depressor body described by Major, Nanninga and Weber [1932] is readily distinguished from the seminal depressor by its stability in boiling alkali and by its not being adsorbed by charcoal. In several ways the seminal depressor is similar to that found by v. Euler and Gaddum

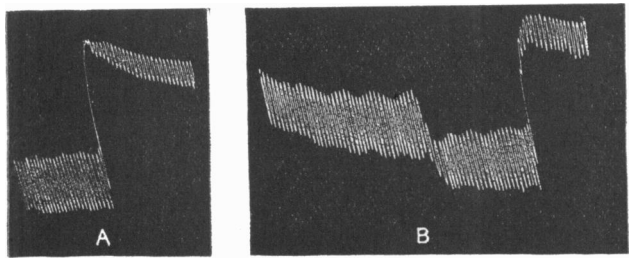


Fig. 2. Rabbit's small intestine. 30 c.c. oxygenated Tyrode. A, 0·1 c.c. seminal extract; B, relaxation due to addition of atropine $1/10^6$ followed by stimulation due to 0·1 c.c. seminal extract.

[1931] in intestinal muscle and brain tissue, but differs from it in not being precipitated by phosphotungstic acid.

It appears, therefore, that considering only its action on the blood-pressure the seminal depressor can be distinguished from all the depressors hitherto described.

### Effect on the isolated intestinal strip.

Both seminal fluid and extracts exert a strong stimulant action on the tone of intestinal muscle. The longitudinal muscle of the rabbit's small intestine was used suspended in a 30 c.c. bath of oxygenated Tyrode solution. It is well known that this preparation is relatively very insensitive to histamine, and its response to choline and its esters is prevented by atropine. Adenosine relaxes the intestinal muscle and the extract of Major *et al.* [1932] has no effect on it.

In Fig. 2 are shown the responses of this preparation to 0·1 c.c. of a seminal extract (representing 0·1 c.c. seminal fluid) before and after the

addition of atropine to the bath. The effect of the extract is to produce a greatly increased tone but not an increased rhythm; the actual excursions are in every case diminished after the increased tone is established. This is rather different from the effect produced by the substance of v. Euler and Gaddum, which always gave a great stimulus to rhythm as well as tone.

Is the effect on the intestinal muscle due to the substance which produces the fall in blood-pressure? Both effects have been demonstrated in the same extracts and ultrafiltrates. Treatment with alkali and acids and then boiling removes almost all the action on the muscle, but not quite so readily as it does that on the blood-pressure. In other ways also the two effects run parallel and it appears reasonable to suppose that only one substance is involved.

### Effect on the virgin uterus.

Seminal plasma and extracts show a powerful oxytocic activity. The uterus of the virgin guinea-pig was used in a 30 c.c. bath of oxygenated Tyrode solution. Assay of the oxytocic activity of seminal extracts in terms of histamine gave values of from 0·4 to 0·6 mg. histamine per c.c. seminal fluid. Similar results were obtained with dialysates and ultrafiltrates. The results of boiling in dilute acid and alkali did not correspond to those of similar treatment on the blood-pressure effects. Boiling for 2 min. in 0·1 *N* NaOH or in 0·1 *N* HCl did not remove the oxytocic effect. Treatment with charcoal hardly removes any of the oxytocic activity of seminal extracts, whereas similar treatment of an appropriate solution of histamine removed about 75 p.c. of the activity of the latter. As far as our experiments go at present it appears that the oxytocic substance in seminal plasma is distinct from the depressor.

### Effect on the isolated seminal vesicle of the guinea-pig.

The seminal vesicle of the guinea-pig, freed from its gelatinous contents and suspended in oxygenated Tyrode solution (30 c.c.), provides an interesting test object, and it seemed relevant to examine the effect of seminal plasma on it.

The seminal vesicles receive their nerve supply from the lumbar and abdominal sympathetic *via* the inferior mesenteric ganglion and from the 3rd and 4th sacral nerves *via* the hypogastric plexus. Ejaculation has been produced by stimulation of fibres from the inferior mesenteric ganglion, and Remey [1884] has described a small ganglion (in the

guinea-pig) close to the inferior vena cava which receives fibres from the renal plexus and gives post-ganglionic fibres to the seminal vesicles and ducts; stimulation of these latter fibres leads to energetic contraction of the vesicles.

It was shown by Waddell [1916] that the freshly excised vasa deferentia of various animals exhibit rhythmic contractions in the usual media and that adrenaline, ergot, pilocarpine, nicotine and $BaCl_2$ increase the tone of this preparation whilst pituitrin produced no effect. He later showed [1917] that the same was true for the excised seminal vesicles or the guinea-pig and the rat, and that atropine antagonized the effects of nicotine and pilocarpine. From these data Waddell concluded that the seminal vesicles of these animals possess a motor sympathetic and a motor parasympathetic supply. More recently

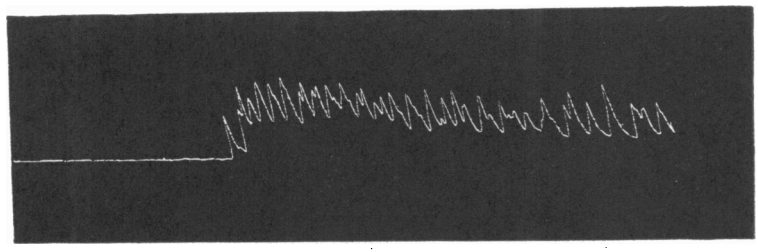

Fig. 3. Isolated seminal vesicle of the guinea-pig. 30 c.c. oxygenated Tyrode. Effect of 0·1 mg. adrenaline on quiescent vesicle.

Bacq [1934] has shown that the seminal vesicle of the guinea-pig is stimulated by adrenaline, but that the effect is poor in the absence of Ca and that ergotamine antagonizes the effect of adrenaline. He also found that acetylcholine produced an effect like that of adrenaline and that it was antagonized by atropine.

In the present experiments, in which Tyrode solution was used, the suspended seminal vesicles did not show marked spontaneous contractions. Fig. 3 shows the effect of 0·1 mg. adrenaline on a quiescent vesicle in 30 c.c. oxygenated Tyrode. Sometimes the effect of adrenaline is to produce an immediate contraction followed by a series of rapid contractions and then a rapid return to the original tone with a slow rhythm of considerable amplitude. The dose of ergotamine tartrate which just fails to inhibit the effect of 0·1 mg. adrenaline is about 0·5 mg. The vesicle preparation is not very sensitive (as seen by the dose of adrenaline required) and rapidly loses its sensitivity after removal from the animal.

The vesicle responds to pilocarpine not by an increase in tone but by the establishment of a rhythm. Pituitrin does not produce any obvious effect. Histamine (0·5 mg. in 30 c.c. bath) produces an effect not unlike that of adrenaline but of very much smaller proportions. Acetylcholine (0·1 mg. in 30 c.c. bath) gives rise to a series of rhythmic contractions but practically no increase in tone; this effect is completely abolished by atropine ($1/10^5$). Adenosine does not have any observable effect.

The effect of seminal plasma or extract is peculiar. If added alone (0·5 and 1·0 c.c. in 30 c.c. bath) to the quiescent vesicle there is sometimes a very slight increase in tone but usually there is no response. If, however, a preliminary dose of adrenaline be added and a considerable interval

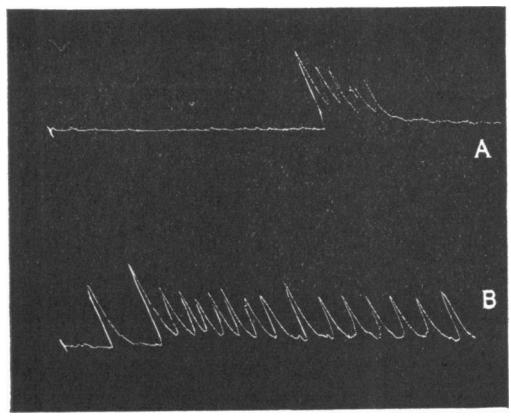


Fig. 4. Isolated seminal vesicle of guinea-pig. 30 c.c. oxygenated Tyrode. A, effect of 0·1 mg. adrenaline; B, effect of 0·5 c.c. seminal extract after that of adrenaline had worn off. The same extract had, in doses of 0·5 and 1·0 c.c., alone produced no effect on the same vesicle.

be allowed to elapse until the only activity of the vesicle is an occasional contraction, then the addition of the seminal extract or plasma is followed by a rapid series of contractions but there is no increase in tone. This effect is shown in Fig. 4. Adrenaline in doses so small that there is hardly any response of the vesicle seems to "potentiate" the vesicle for seminal extracts. The hypothesis that there may be a synergism between adrenaline and seminal plasma in bringing about an effective evacuation of the vesicles suggests itself, but as the contents of the seminal vesicles of the guinea-pig do not, in fact, possess any action on the isolated vesicle we must simply consider this effect of seminal extracts as another example of their power to stimulate smooth muscle, and that in the case of the

vesicle a preliminary stimulus is necessary to produce a response sufficient to be recorded. Whilst we have obtained this effect with the seminal fluid of a vasectomized man it is not possible as yet to say whether the active substance arises from the prostate or the seminal vesicles or both.

Whether effects of this kind can be interpreted in relation to the functions of the accessory genital organs of the male is doubtful. v. Euler [1934] has extracted from the seminal vesicles of some animals substances with an inhibitory action on smooth muscle and resembling adenylic acid, and also others with a stimulant action like that described above for seminal plasma. In regard to these effects of seminal plasma it is not easy to see how the active substance can readily get into intimate contact with the relevant smooth muscle.

### Effect on the eserinized rectus abdominis of the frog.

Although the evidence already given makes it improbable that choline or choline esters are present in seminal plasma, such evidence is only significant in relation to the delicacy of the methods used for the detection of these bodies. The eserinized rectus abdominis of the frog is a test object which combines great sensitivity with rapidity of response when dealing with acetylcholine. The recommendations of Chang and Gaddum [1933] were followed in the use of this preparation and their finding confirmed that the response to KCl or choline is not much affected by preliminary treatment with eserine, whilst that to acetylcholine is very greatly increased by such treatment. After some failures preparations could be made which responded immediately to 1$\gamma$ of acetylcholine. Fig. 5 shows the result of an experiment to compare the effects of seminal extract and acetylcholine. The magnification was about 6 and the load 3 g. After each contracture the bath was changed and eserine added afresh; the kymograph was stopped during relaxation. Although Chang and Gaddum [1933] recommend the addition of eserine after each change of the bath fluid, we find the preparation may remain equally sensitive to acetylcholine for as long as 4 hours with frequent changes without repeating the addition of eserine.

Since seminal extracts have very little effect on the noneserinized muscle and, as seen in Fig. 5, a marked effect on the eserinized one it is justifiable to speak of an acetylcholine equivalent of seminal plasma in the sense proposed by Chang and Gaddum. Expressed in this way the acetylcholine equivalent of seminal plasma is not more than 1$\gamma$ per c.c. Considering the values obtained by the above authors for the majority of organ extracts they studied, the acetylcholine equivalent of seminal

plasma must be regarded as high. It is highly improbable that the substance responsible for this effect in seminal plasma is acetylcholine, first because the fluid (alkaline) is not received until some 10 hours after ejaculation, secondly because the latent period of the effect on the rectus is too long (30 sec. in Fig. 5), and lastly because even in extracts with an acetylcholine equivalent of 1$\gamma$ per c.c. the effect on the isolated intestine is absolutely unaffected by atropine.

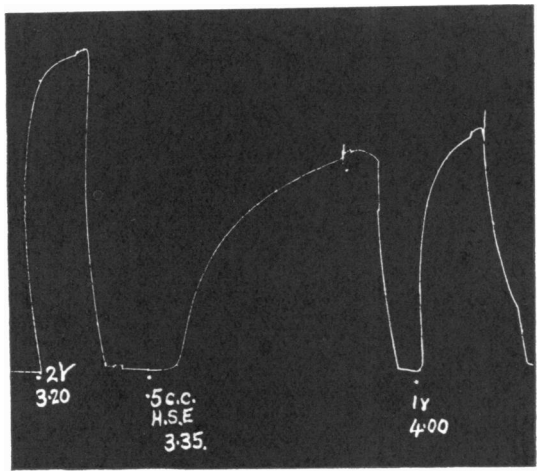


Fig. 5. Eserinized rectus abdominis of the frog. 20 c.c. Ringer. Eserine $1/10^5$. From left to right the contractures are due to the addition of 2$\gamma$ acetylcholine, 0·5 c.c. seminal extract and 1$\gamma$ acetylcholine.

## Discussion.

The existence of substances with marked pharmacological properties in tissues is well known, but their function is often not clear. The presence in human seminal fluid of a substance producing strong vasodilatation and stimulation of plain muscle is perhaps easier to understand than in most other situations.

The origin of the seminal depressor is not clear. Extracts prepared from enlarged (adenomatous) prostates, removed at operation, do not contain the active substance dealt with above. An enlarged prostate weighing 75 g. was treated in the same way as seminal plasma and yielded 80 mg. of a waxy extract. This extract was freely soluble in water but it had no effect on blood-pressure even in large doses; it had, however, a marked inhibitory effect on the isolated atropinized intestinal strip. v. Euler [1934] states that a body identical with the P substance

described by Euler and Gaddum is present in human prostates obtained post-mortem and from the seminal vesicles of sheep and pigs; but in a private communication he casts doubts upon the identity of these two substances.

We have sought for the active seminal depressor in the seminal fluid of the horse (kindly supplied by Dr Walton, Agricultural Laboratory, Cambridge) and failed to find it. It appears also to be absent from the fluid obtained from rabbit's testes and epididymis and from the contents of the seminal vesicles of the rat and the guinea-pig. On the other hand, v. Euler [1934] finds it in the extract of the rabbit's prostate and, in small amounts, in that of the guinea-pig.

It is impossible to state whether the seminal depressor has any universal function. It seems improbable that it is simply an excretory product without physiological function. The composition of seminal plasma is so peculiar when compared with other body fluids and so specially adapted to the functions of nutrition and mobilization of the sperm cells that the presence of a substance with marked pharmacological properties can scarcely be regarded as fortuitous. It may be remarked here that the seminal extracts used contained no spermine, which in any case is without any obvious pharmacological effect on the test objects used.

The presence in seminal extract of small amounts of proteose led us to see if the depressor is destroyed by trypsin; it is, however, quite unaffected by such treatment. The probabilities are that the active substance is of relatively simple structure, and that its principal use in the organism is to ensure a considerable local vaso-dilatation and hence adequate gaseous exchange and secretory activity in the accessory glands of the male. Provided there is some way in which the active substance can come into contact with the muscle of the genital tract, it is possible that it may contribute to the maintenance of the peristalsis incident to ejaculation.

With regard to the effect on the rectus abdominis there is no question of it being due to potassium or choline. The enormously increased effect after adding eserine is paralleled only by the similar response to acetylcholine. All that can be said at present is that seminal plasma contains a substance with an action on this preparation like that of acetylcholine [cf. Chang and Gaddum, 1933].

SUMMARY.

1. Human seminal plasma has been shown to contain a powerful vaso-dilator and to produce strong stimulation of plain muscle. The substance responsible for these effects is distinguishable from other tissue extracts having similar properties. It is suggested that the vaso-dilator may be of importance in the maintenance of local vaso-dilatation in the accessory glands and ducts of the male genital tract and so ensure an active gaseous exchange between the blood and the various seminal secretions.

2. It is probable that the oxytocic power of the seminal plasma is due to a substance distinct from the depressor. The histamine equivalent of the seminal oxytocic substance is between 0·4 and 0·6 mg. per c.c.

3. Seminal plasma contains a substance with an effect on the eserinized rectus abdominis of the frog like that of acetylcholine. The acetylcholine equivalent of seminal plasma is not greater than 1$\gamma$ per c.c.

REFERENCES.

Bacq, Z. (1934). *Arch. intern. Pharmacodyn.* **47**, 123.
Chang, H. C. and Gaddum, J. H. (1933). *J. Physiol.* **79**, 255.
v. Euler, U. S. (1934). *Arch. exp. Path. Pharmak.* **175**, 78.
v. Euler, U. S. and Gaddum, J. H. (1931). *J. Physiol.* **72**, 74.
Frey, E. K., Kraut, H. and Schultz, F. (1930). *Arch. exp. Path. Pharmak.* **158**, 334.
Goldblatt, M. W. (1933). *Chem. and Indust.* **52**, 1056.
Major, R. H., Nanninga, J. B. and Weber, C. J. (1932). *J. Physiol.* **76**, 487.
Remey, C. (1884). *C. R. Soc. Biol.*, Paris, **1**, 497.
Waddell, J. A. (1916). *J. Pharmacol.*, Balt., **8**, 551.
Waddell, J. A. (1917). *Ibid.* **9**, 113.